AD_____

AWARD NUMBER: W81XWH-14-1-0255

TITLE: BIOENERGETICS OF STROMAL CELLS AS A PREDICTOR OF AGGRESSIVE PROSTATE CANCER

PRINCIPAL INVESTIGATOR: Praveen K. Vayalil

RECIPIENT: University of Alabama at Birmingham
Birmingham, AL 35294

REPORT DATE: September 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2015	Annual	1 Sep 2014 - 31 Aug 2015

4. TITLE AND SUBTITLE

Bioenergetics Of Stromal Cells As A Predictor Of Aggressive Prostate Cancer"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0255

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Praveen K. Vayalil PhD

E-Mail : praveen@uab.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Alabama at
Birmingham, Birmingham-35294

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Mitochondrial function is influenced by alterations in oncogenes and tumor suppressor genes and changes in the microenvironment occurring during tumorigenesis. Therefore, we hypothesized that mitochondrial function will be stably and dynamically altered at each stage of the prostate tumor development. We tested this hypothesis in RWPE-1 cells and its tumorigenic clones with progressive malignant characteristics (RWPE-1<WPE-NA22<WPE-NB14<WPE-NB11<WPE-NB26) using high-throughput respirometry. Our studies demonstrate that mitochondrial content do not change with increasing malignancy. In premalignant cells (WPE-NA22 and WPE-NB14), OXPHOS is elevated in presence of glucose or glutamine alone or in combination compared to RWPE-1 cells and decreases with increasing malignancy. Glutamine maintained higher OXPHOS than glucose and suggests that it may be an important substrate for the growth and proliferation of prostate epithelial cells. Glycolysis significantly increases with malignancy and follow a classical Warburg phenomenon. Fatty acid oxidation (FAO) is significantly lower in tumorigenic clones and invasive WPE-NB26 does not utilize FAO at all. In this paper, we introduce for the first time the mitochondrial oncobioenergetic index (MOBI), a mathematical representation of oncobioenergetic profile of a cancer cell, which increases significantly upon transformation into localized premalignant form and rapidly falls below the normal as they become aggressive in prostate tumorigenesis. We have validated this in five prostate cancer cell lines and MOBI appears to be not related to androgen dependence or mitochondrial content, but rather dependent on the stage of the cancer. Altogether, we propose that MOBI could be a potential biomarker to distinguish aggressive cancer from that of indolent disease.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Cancer cells and cancer associated fibroblasts (CAFs) acquire distinct bioenergetic characteristics as they tumor progresses from indolent to aggressive form under different oxygen concentrations. CAFs and cancer cells also mutually regulate their bioenergetics for the growth and progression of the disease under different oxygen tensions. These characteristic bioenergetic behaviors of these cells can be easily measured by detailed bioenergetic profiling using XF-Analyzer. These differences obtained by metabolic phenotyping of cancer and CAFs may have significant prognostic value to distinguish aggressive prostate cancer (PCa) from indolent tumor

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Mitochondria, bioenergetics, oncobioenergetics, prostate cancer, indolent tumor, aggressive tumor, XF analyzer

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Establish optimal XF analyzer conditions for the stress tests in cells in cultures e
2. Establish fundamental bioenergetic characteristics of CAFs, PFs, and PC cells
3. Establish fundamental bioenergetic characteristics of CAFs, PFs, and PC cells at different oxygen tensions
4. Establish the influence of PC cells on the bioenergetic parameters of CAFs and PFs under normoxic and hypoxic conditions
5. Establish the influence of CAFs and PFs on the bioenergetic parameters of PC cells under normoxic and hypoxic conditions
6. Establish the role of CAFs and PFs secreted metabolites in regulating the cancer cell bioenergetics under normoxia and de-oxygenation and re-oxygenation conditions.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities: Cancer cell lines (RWPE-1 cells and its tumorigenic clones), fibroblasts (WPMY-1) and reagents and kits were procured from vendors and cultures were established in the laboratory. Enough vials of stock cultures were saved for future use. XF-assay was optimized for each cell line. Bioenergetics of each cell line was measured at ambient oxygen tension.

Specific Objectives:

1. Determine the bioenergetic profile of prostate cancer cell lines at ambient O_2 Tension
2. Determine the substrate-dependent bioenergetics of Prostate cancer cell lines
3. Define oncobioenergetic index of prostate cancer cells

Significant Results:

Introduction:

Mitochondrial function is influenced by alterations in oncogenes and tumor suppressor genes and changes in the microenvironment occurring during tumorigenesis. Therefore, we hypothesized that mitochondrial function will be stably and dynamically altered at each stage of the prostate tumor development.

Methodology:

We tested this hypothesis in RWPE-1 cells and its tumorigenic clones with progressive malignant characteristics (RWPE-1<WPE-NA22<WPE-NB14<WPE-NB11<WPE-NB26) using high-throughput respirometry.

Extracellular flux (XF) analyzer (Seahorse, Billerica, MA) was used to measure the mitochondrial oncobioenergetics. XF analyzer simultaneously measures in real-time the two major energy yielding pathways-cellular aerobic respiration and glycolysis. It monitors O_2 consumption rate (OCR; due to mitochondrial respiration) and extracellular acidification rate (ECAR; predominantly due to glycolysis) in real-time. All the cells are subjected to three bioenergetic tests – mitochondrial and glycolytic stress test (MiST and GlyST). Cells were plated at an optimal cell density which was determined for each cell line before the analysis and found to be 3×10^4 cells/well for XF^e96 analyzer.

Results:

1. Our studies demonstrate that mitochondrial content do not change with increasing malignancy Figure-1. Mitochondrial content was demonstrated by the citrate synthase activity/abundance (a housekeeping mitochondrial enzyme) and abundance was normalized to mitochondrial membrane protein VDAC.

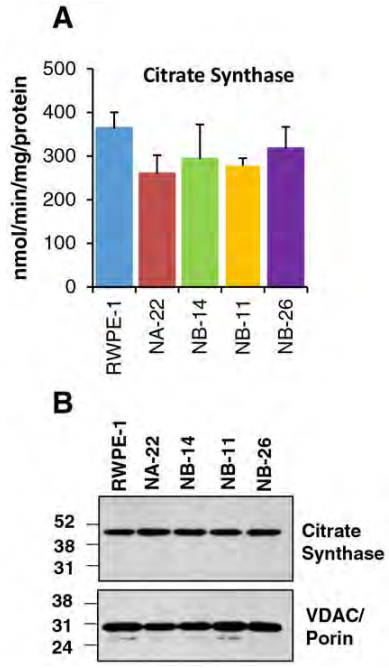

Figure 1: Mitochondrial content in RWPE-1 and it tumorigenic clones. Citrate synthase enzyme activity **A**. Cells were lysed in a lysis buffer containing protease inhibitors. The data is represented as nmol/min/mg protein. The values are mean ± SE of two separate experiments performed in triplicates. Abundance of citrate synthase and VDAC/porin proteins in prostate epithelial cell lysate **B**. Total proteins isolated from cells were blotted with antibodies for citrate synthase and VDAC/porin. A representative image from two separate western blots is shown.

2. In premalignant cells (WPE-NA22 and WPE-NB14), OXPHOS is elevated compared to RWPE-1 cells and then decreases with increasing malignancy in presence of glucose or glutamine alone or in combination. It was performed using MIST.

MiST: After three baseline OCR measurements in an assay medium (DMEM containing 10 mM glucose, 4 mM glutamine at pH 7.4 without bicarbonate), Oligomycin (1.0 µM), FCCP (0.125 µM), and antimycin A (10 µM) were injected sequentially with OCR and ECAR measurements recorded after each injection. The following mitochondrial respiratory functional characteristics were elucidated from the OCR trace: basal OCR (OCR in the absence of any mitochondrial inhibitors), ATP-dependent OCR (OCR necessary to synthesize ATP), reserve capacity (difference between the maximal and basal OCR), which is an estimate of the potential bioenergetic reserve the cell can call upon in times of stress, non-mitochondrial (OCR after the addition of Antimycin A) and proton leak. Glucose –, glutamine –, and FA – dependent MiST were performed using an assay medium only containing glucose (10 mM), glutamine (4 mM) or palmitate-BSA (0.17 mM) as substrate for energy production.

Basal = OCR without inhibitors − Antimycin inhibted OCR Eq1

Maximal = OCR due to FCCP − Antimycin inhibited OCR Eq2

Reserve Capacity = Maximal − Basal Eq3

Proton Leak = Oligomyin Inhibited OCR − Antimycin inhibited OCR Eq4

Non − mitochondrial = Antimycin Inhibited OCR Eq5

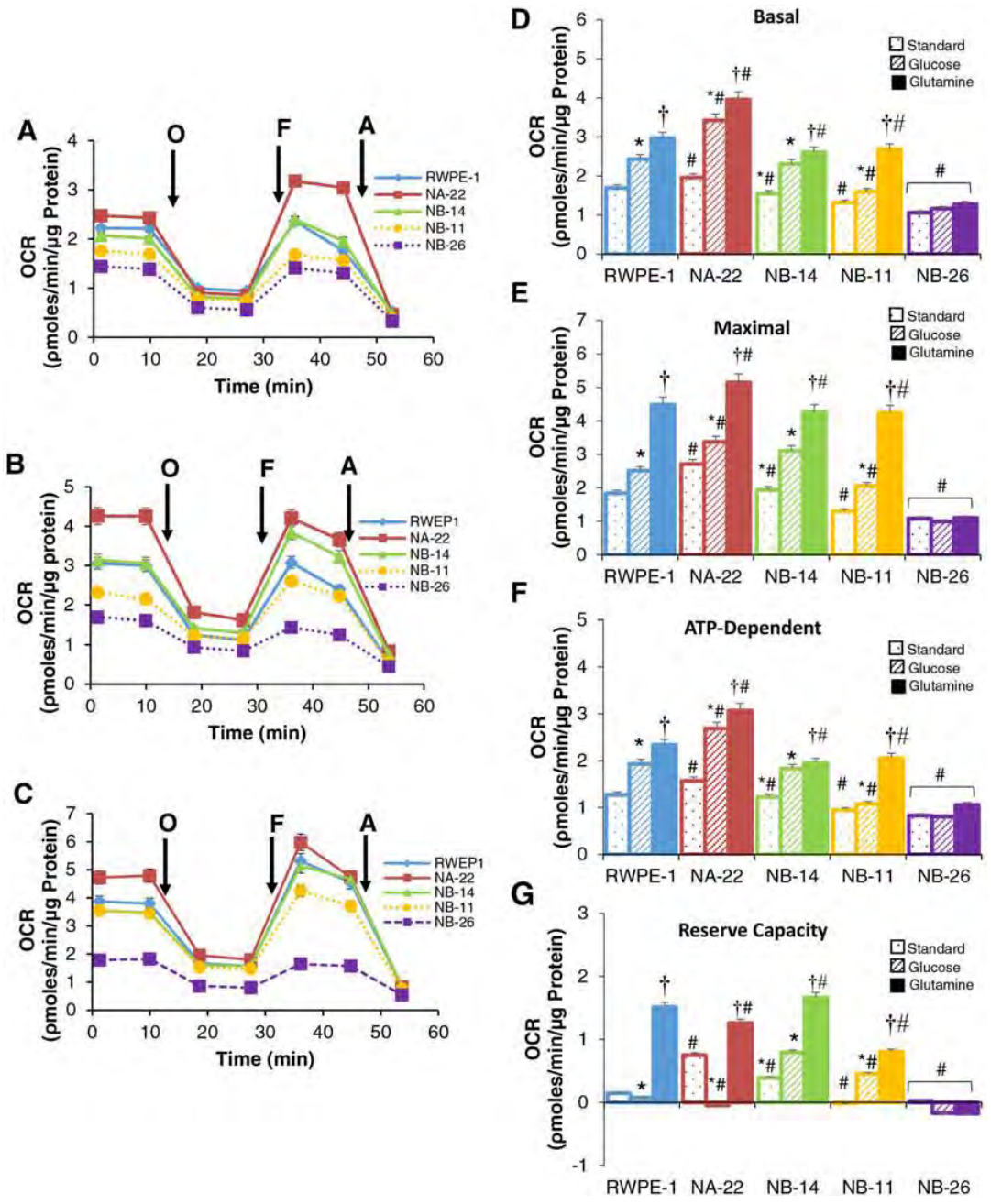

Figure 2: Oncobioenergetic profile of RWPE-1 and its clones analyzed by MiST in standard or substrate limited assay media. Cells were plated on XF96 plates in standard XF assay media. The medium was removed and replaced with **A.** standard XF assay medium (DMEM, supplemented with 5.0 mM glucose and 4.0 mM glutamine without bicarbonate (pH 7.4)), or **B.** DMEM with glucose (5.0 mM) or **C.** glutamine (4 mM) as the only energy substrate and equilibrated 1 h before MiST. OCR of different cell lines was plotted against time after sequential injection of oligomycin (O); FCCP (F); and antimycin (A) Values are the mean ± SE of observations made from 15–30 wells from two separate experiments [♦RWPE-1; ■ WPE1-NA22; ▲ WPE1-NB14; ● WPE1-NB11; ■ WPE1-NB26]. Major oncobioenergetic parameters of RWPE-1 and it tumorigenic clones in presence of different energy sources **D–G.** Cells provided with standard (dotted) or in glucose (diagonal striped) or glutamine (solid filled) limited assay medium. Basal (D), maximum (E) ATP-dependent/oligomycin inhibitable (F) respiration and reserve capacity (G) were calculated from the OCR traces corresponding to each substrate as depicted in A-C. Values are the mean ± SE of observations made from 15–30 wells from two separate experiments. *$p < 0.05$ compared to standard assay media, † $p < 0.05$ compared to standard assay media and glucose restricted media; #$p < 0.05$ compared to RWPE-1 cells or WEP1-NA22

exposed to corresponding substrate.

3. Glycolysis significantly increases with malignancy. Glycolytic parameters such as glycolytic rate (ECAR in the presence of glucose), glycolytic capacity (maximum attainable glycolysis), and glycolytic reserve (difference between glycolytic capacity and glycolysis) were determined from the ECAR trace (Fig 1A). It was performed using GlyST (see figure legend for details).

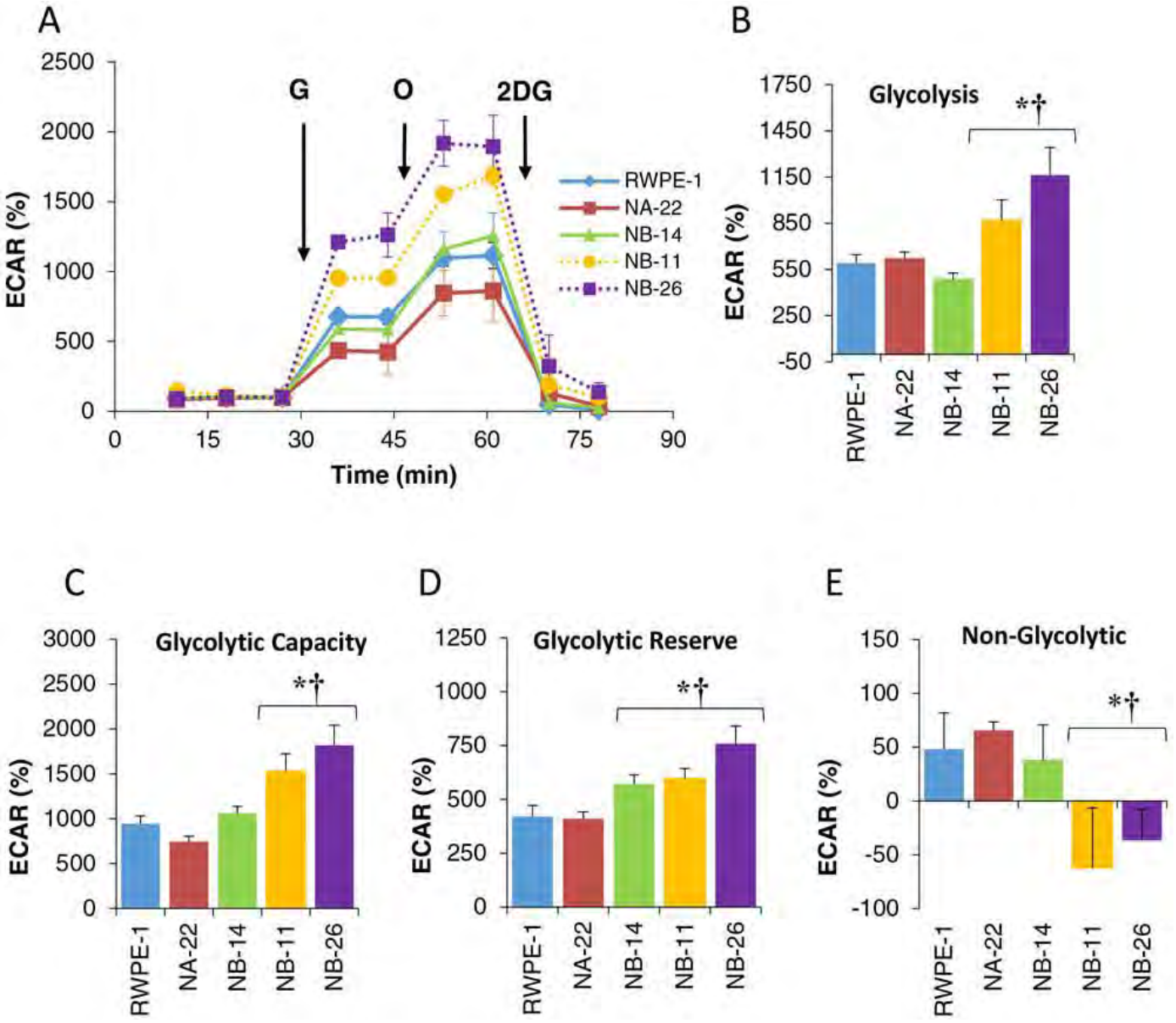

Figure 5: Glycolytic profile of RWPE-1 and its clones analyzed by GlyST. Cells were plated on XF96 plates in complete media. After 24 hr. the media was changed to an assay medium containing 2 mM glutamine without glucose for 1.0 hr. After three ECAR measurements without glucose, 10 mM glucose (final), Oligomycin (1.0 μM final) and 2DG (100 mM final) were added sequentially. The resulting ECAR trace was normalized to the baseline (ECAR without glucose) represented in percent and was used to calculate various glycolytic parameters of each cell. The values are mean ± SE of 12 to 30 wells from two separate experiments. *$p < 0.05$ compared to RWPE-1 and † compared to WPE1-NA22.

4. We introduced for the first time the mitochondrial oncobioenergetic index (MOBI), a mathematical representation of oncobioenergetic profile of a cancer cell, which increases significantly upon transformation into localized premalignant form and rapidly falls below the normal as they become aggressive in prostate tumorigenesis (**Figure 4**). In addition to RWPE-1 cells and its clones, we have further validated this in five prostate cancer cell lines (three cell lines (OPCT-1, −2 and −3) isolated from localized prostate cancer of stage T1c, T2a and T3a with Gleason score ≥6 respectively and well established metastatic cell lines, DU145 (brain) and LNCaP (lymph node)) and MOBI appears to be not related to androgen dependence or mitochondrial content, but rather dependent on the stage of the cancer. Altogether, we propose that MOBI could be a potential biomarker to distinguish aggressive cancer from that of indolent disease. MOBI was calculate using the following equation

$$Basal\ OCR \times \log\left[\frac{Reserve\ capaity + ATP\ Dependent}{Proton\ Leak + Non - mito.OCR}\right]$$

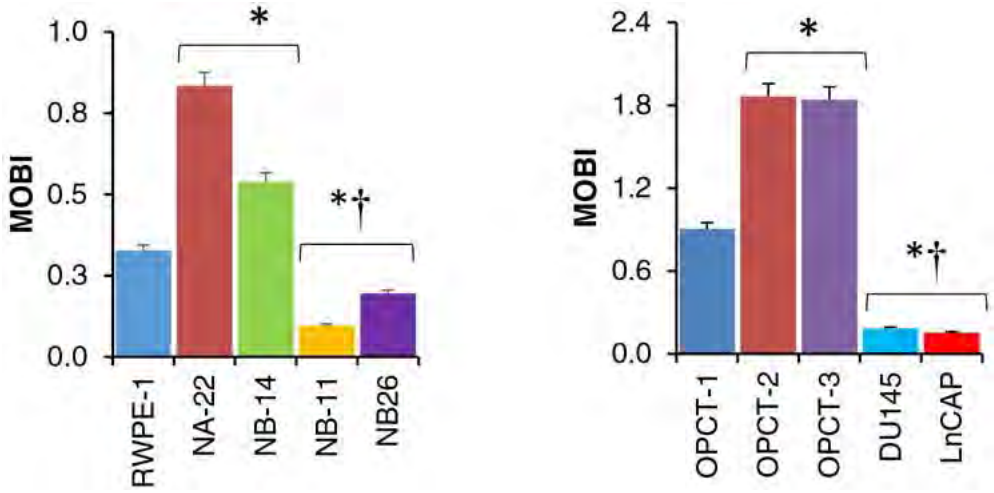

Figure 4: Mitochondrial Oncobioenergetic index of prostate epithelial cells. MOBI of RWPE-1 and its tumorigenic clones **A.** and few commercially available prostate cancer cell lines of various degrees of malignancy **B.** was calculated from the oncobioenergetic parameters obtained from OCR trace for each cell line.

Other Achievements:

1. Presented at AACR meeting

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The effects of different O2 tension on the bioenergetic function are being studied in PCa cells. We will also study the effects of different O2 tension on CAFS and normal PFs on their bioenergetics. Co-culture studies with PCa and CAFS are also under progress. This will accomplish the specific aim-2 of the proposed project.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

One of the major clinical problems in newly diagnosed men with prostate cancer is to distinguish aggressive prostate cancer from indolent disease. As an attempt to use mitochondrial oncobioenergetics as a potential marker for tumor aggressiveness, we introduced the concept of MOBI in prostate cancer. We hypothesized that as the normal cell transforms into a pre-malignant cell and progresses through different stages into aggressive tumor, oncobioenergetics at each stage will also change correspondingly to support the growth of tumor cells. This work provides for the first time a mathematical analysis of mitochondrial oncobioenergetic profile of cancer cells that could be related to the aggressiveness of prostate cancer. We demonstrated that as the normal cells transform into localized premalignant tumor, they tend to have a very high MOBI than the normal cells and as the cells achieve invasive properties, the MOBI falls far below the MOBI of normal cells. Changes in MOBI occur irrespective of their differences in the individual oncobioenergetic parameters or androgen requirement. Although, high-throughput respirometry is not a feasible approach to determine the mitochondrial function in vivo, certain novel imaging techniques to determine various parameters of mitochondrial function would be a novel approach for translational studies to distinguish aggressive prostate cancer. In conclusion, these results demonstrate for the first time that mitochondrial oncobioenergetic signature of prostate epithelial cells stably and gradually changes at each stage of the tumor progression and MOBI could be a potential biomarker to distinguish the aggressive phenotype from indolent one.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *Adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *Improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication*

(published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Bioenergetic signature of stromal cells defines the aggressiveness of prostate cancer
PK Vayalil, A Landar; Cancer Research 75 (15 Supplement), 3054-3054

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*

- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.